

CANADIAN JOURNAL OF ANIMAL SCIENCE

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No. 1

PROTEIN STORAGE OF PIGS IN THE ABSENCE OF BODY WEIGHT GAIN¹

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[Received for publication January 27, 1960]

ABSTRACT

Five- to six-month-old pigs were maintained at constant weight for 6-week periods on rations containing 16, 26 and 33 per cent protein. Results of 72 7-day nitrogen balance trials showed that pigs could retain up to 6.5 grams of nitrogen per day for 6 weeks. Highest nitrogen retention was obtained on the 26 and 33 per cent protein rations with little difference between the maximum retention figures at either level. A preliminary carbon-nitrogen balance indicated that there was little change in the fat stores of the pigs.

INTRODUCTION

It is well known that the ratio of fat to protein deposited by growing pigs increases with age and that by partially restricting the energy intake this ratio may be reduced (8). Animals with low fat reserves such as calves retain little or no nitrogen when the energy intake is reduced to a level that will just maintain body weight (1, 2). Experiments with obese humans, however, have demonstrated that, with proper diets, considerable body fat may be lost while nitrogen balance is positive (7). The ratio of fat to protein deposited by market weight pigs under severe energy restriction has received very little attention. The market weight pig not only has large fat stores but is still actively growing and it would appear possible that, with a restricted consumption of a high protein ration, considerable protein could be stored in the absence of fat deposition.

Such a regime may not be practical for commercial pig raising, but was tested in the present study, using energy and nitrogen balance techniques to learn more about the fundamental principles governing protein storage.

EXPERIMENTAL PROCEDURE

Male Yorkshire x Landrace pigs, self-fed a 14.5 per cent protein ration from 50 kilograms until 74 to 97 kilograms in body weight, were placed on experiment in groups of four. Group 1 received Ration 1 (Table 1) at an initial level of 13.5 grams per kilogram of body weight per day. This was estimated to supply approximately 40 digestible calories per kilogram of body weight per day, slightly above the resting maintenance requirement given by Brody (3). The pigs were placed in tared metal cages and weighed. They were weighed again 2 days later and twice weekly thereafter. The level of feeding was adjusted as the experiment progressed so as to maintain body weight as close as possible to that recorded after 2 days,

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TABLE 1. — COMPOSITION OF THE EXPERIMENTAL RATIONS

	Ration 1	Ration 2	Ration 3
		(per cent)	
Oats.....	40	22	30
Barley.....	40	39	25
Soybean oil meal (44%).....	4	25	39
Meat meal (50%).....	6	7	7
Fish meal (63%).....	6	8	8
Dried brewers' yeast.....	3	—	—
Iodized salt.....	1	1	1
Vitamins ¹			
Moisture.....	10.06	11.69	10.69
Protein (N x 6.25).....	16.58	26.25	33.31
Ether Extract.....	4.43	2.60	2.66
Ash.....	6.51	7.41	8.39
Fibre.....	7.82	8.64	7.36
NFE.....	54.60	43.41	37.59
Calories per gm. D.M.....	4.45	4.46	4.41

¹Vitamins: 4000 I.U. vitamin A palmitate and 800 I.U. vitamin D₃ per kg. of all rations. B vitamins: 3.8 mg. thiamine, 7.5 mg. riboflavin, 37.5 mg. niacin and 33.8 mg. calcium pantothenate per kg. Rations 2 and 3.

that is, after the initial shrinkage which resulted from the reduced level of feeding. The feces and urine of each pig were collected continuously from the second to forty-fourth days with the collection divided into six 7-day periods. The feces of each pig were collected twice daily, placed in a polyethylene bag, and frozen. They were later weighed, partially thawed and mixed in a Hobart food cutter*. Triplicate samples, weighing from 150 to 200 grams were removed and analysed for nitrogen in a macro-kjeldahl apparatus, using mercury as a catalyst. Dry matter determinations were made in triplicate on 150- to 200-gram samples. These were dried at 100°C. in a forced air oven, weighed, combined, ground in a Wiley mill, and analysed for nitrogen, ash, ether extract and crude fibre. Nitrogen-free extract was obtained by difference. Each ration was mixed in one batch, sampled and analysed for the proximate constituents. Energy determinations were made on feed and feces using a Parr oxygen bomb. The urine was collected in sufficient HCl to maintain a pH below 4. A 5 per cent sample was withdrawn and analysed for nitrogen.

The same procedure was repeated with a second group of four pigs, except that the protein level of the ration was raised to 26 per cent (Ration 2) and the level of feeding required for maintenance was adjusted according to information obtained with Group 1. A third group of four pigs was later used to compare levels of 26 and 33 per cent protein (Rations 2 and 3).

An estimate of fat balance in the body was obtained by running a carbon-nitrogen balance during the eighth week with one of the pigs receiving the 33 per cent protein ration. Expired carbon dioxide was measured by placing the pig, while still in its metal collection cage, in a respiration chamber for two 24-hour periods. The average daily nitrogen and carbon content of the food, feces and urine were determined for the 7-day period.

*Hobart Mfg. Co., Toronto, Ont. Model 84141.

TABLE 2. — AVERAGE DIGESTIBLE CALORIC INTAKE, BODY WEIGHT AND NITROGEN RETENTION FOR EACH PERIOD¹

Period	Body wt. ² / (kg.)	N balance mg./kg./day	Dig. Cal./day	Body wt. (kg.)	N balance mg./kg./day	Dig. Cal./day
GROUP 1 (16% protein)			GROUP 2 (26% protein)			
1	92.4	53.4	41.3	81.0	125.4	32.7
2	92.6	37.7	40.1	80.8	82.8	32.7
3	92.9	24.1	39.2	80.9	67.8	33.2
4	95.0	18.9	36.5	80.4	43.5	30.6
5	95.5	8.3	35.2	80.3	22.5	30.6
6	96.3	32.6	33.2	79.6	30.1	33.7
GROUP 3 (26% protein)			GROUP 3 (33% protein)			
1	77.1	63.2	31.4	76.3	90.7	31.6
2	76.1	33.9	31.4	75.8	60.6	32.0
3	75.8	53.6	32.7	75.9	78.5	32.6
4	75.2	63.6	34.8	76.0	90.9	34.4
5	75.9	76.6	34.3	76.0	72.0	32.6
6	76.4	50.1	34.3	76.2	60.5	33.0

¹ Four pigs per group with two pigs on each level of protein in Group 3² Body weight recorded at the beginning of each period

RESULTS AND DISCUSSION

The average weekly results for each treatment are shown in Table 2 and the cumulative 6-week results for each pig are given in Table 3. The initial estimate of approximately 40 digestible calories per kilogram per day for maintenance was found to be high. The pigs in Group 1 began to gain weight at this level and the feed allowance was reduced weekly until a level of 33.2 digestible calories was reached in Period 6. The pigs gained an average of 4.2 kilograms during the 6 weeks and retained an average of 108 grams of nitrogen.

A level of 33.0 digestible calories per kilogram was found to be optimum for maintenance of body weight in Groups 2 and 3. Group 2, fed Ration 2 (26 per cent protein), lost an average of 1.2 kilograms in body weight over the 6 weeks but retained an average of 209 grams of nitrogen per pig. Two pigs which did not lose weight (No. 5 and No. 7) retained an average of 264 grams of nitrogen. Pigs 9 and 11 in Group 3, also fed Ration 2, retained an average of 182 grams of nitrogen while pigs 10 and 12, fed Ration 3 (33 per cent protein), retained an average of 241 grams of nitrogen. Ration 3 produced a significantly greater ($P < 0.01$) nitrogen retention than Ration 2 when individual periods in Group 3 (Table 2) were compared. The pigs in Groups 2 and 3 consumed an average of 827 grams of feed and retained up to 6.5 grams of nitrogen per day for 42 days. This may be compared with work of Lassiter *et al.* (6) in which growing pigs of approximately the same weight consumed 2.0 kilograms of 10, 14 and 16 per cent protein rations daily and retained 10, 16 and 18 grams of nitrogen, respectively.

The total protein that a pig can store in the absence of body weight gain obviously must have some limit. All pigs were still in positive nitrogen

TABLE 3. — CUMULATIVE 6-WEEK BALANCE FOR EACH PIG

Pig No.	Initial wt. (kg.) ¹	Total wt. gain (kg.)	Total dig. therms	Total N intake (gm.)	Total N balance (gm.)
Group 1, Ration 1					
1	88.0	6.3	152	1388	179
2	94.1	2.7	147	1401	32
3	94.3	5.0	144	1400	126
4 ²	93.3	3.0	147	1401	96
Av.	92.4	4.2	147	1397	108
Group 2, Ration 2					
5	77.6	0.0	98	1304	258
6	83.7	-3.6	111	1419	178
7	84.4	0.2	118	1531	271
8 ³	78.2	-1.6	105	1383	129
Av.	81.0	-1.2	108	1409	209
Group 3, Ration 2					
9	80.7	-0.1	114	1596	201
11	73.5	0.2	98	1401	164
Av.	77.1	0.0	106	1498	182
Group 3, Ration 3					
10	78.2	1.1	110	1904	259
12	74.6	0.1	99	1742	224
Av.	76.3	0.6	104	1823	241

¹ Initial weight is that recorded after first 2 days on restricted intake of experimental rations.

² Time on experiment was limited to 41 days owing to a 6-day collection in Period 6.

³ Time on experiment was limited to 41 days, owing to a 6-day collection in Period 1.

balance at the end of 6 weeks but nitrogen retention equal to that recorded during the first periods was only attained at higher caloric intakes (Table 2). In some cases the pigs were recovering weight that had been lost during the earlier periods. The average 6-week nitrogen retention of 241 grams recorded by pigs 10 and 12 is equivalent to an increase of 1.51 kilograms in body protein. This is equal to an increase of 15.2 kilograms in body weight or 20.0 per cent increase in body protein, assuming a normal carcass composition of 9.9 per cent protein (8). It is well known that the body composition of pigs may vary greatly depending upon the dietary level of protein and energy. Jordan (5) found that the carcasses of pigs restricted to a "70 per cent corn ration" on pasture contained 20.4 per cent more protein than those of pigs fed a "full corn ration". The accuracy of using nitrogen balances in determining protein storage was shown by the work of Harnisch and Becker (4) in which the average nitrogen retention of pigs in two 20- to 25-day trials was within 0.3 and 0.5 per cent of that

TABLE 4. — AVERAGE APPARENT DIGESTIBILITY COEFFICIENTS OF THE RATIONS

Pig No.	Ration 1	Ration 2		Ration 3
	1, 2, 3, 4	5, 6, 7, 8	9, 11	10, 12
Dry matter	71.3	72.5	73.4	75.3
Protein	73.5	85.5	85.8	89.1
Fat	87.0	85.4	85.2	86.0
Ash	40.5	46.9	49.0	43.6
Fibre	21.7	17.0	31.0	32.4
NFE	79.5	76.0	76.8	76.3
Calories	72.5	74.6	75.2	77.6

determined from carcass analysis. The present experiment demonstrates that the change in body composition of pigs does not necessarily have to take place while the pig is growing but may be changed while the body weight remains constant.

Digestibility

The digestibility of the rations is shown in Table 4. There was a significant increase ($P > 0.01$) in apparent protein digestibility with each increase in protein content. Differences in apparent protein digestibility may sometimes result from the excretion of metabolic fecal nitrogen which is equivalent to a greater percentage of dietary nitrogen on low than on high protein diets. Whiting and Bezeau (10) reported that metabolic fecal nitrogen excretion of 65 kilogram pigs averaged 0.26 grams per 100 grams of dry matter intake (5 per cent fibre). In the present experiment, total fecal nitrogen excretion of pigs fed 16, 26, and 33 per cent protein rations averaged 0.81, 0.66 and 0.65 grams respectively per 100 grams of dry matter intake. No matter what figure is used for metabolic fecal nitrogen (based on body weight, dry matter, or fibre intake) the digestibility of true protein increases with each increment in dietary protein. This would suggest a higher digestibility of the protein supplement which made up a greater proportion of the high protein ration. The increase in protein digestibility was also reflected in an increase in dry matter and energy digestibility.

Body Protein, Water, Fat Relationships

The nitrogen balance data indicated an increase in the protein content of the pigs but did not show if this increase took place at the expense of body fat or water. The carbon-nitrogen balance obtained with pig 12 indicated that the pig was not losing fat but was actually in a slight positive balance. There was an estimated daily gain of 33.1 grams fat, 37.2 grams protein, and 50 grams of body weight. The muscle tissue of pigs contains approximately 75 per cent water, the percentage decreasing with age (8). None of the pigs could have retained water in the same ration to nitrogen as that found in the body without gaining weight or losing a considerable amount of fat. Such a large loss of fat (approximately 150 grams per day) would have been readily detected by the carbon-nitrogen balance. Even with small losses in body fat, there would still be an increase in the ratio of tissue nitrogen to tissue water.

The alteration of the protein content of pigs while maintained at constant body weight indicates the strong tendency of pigs to grow under conditions of severe caloric restriction. It is possible that, with a small increment in energy allowance above maintenance, considerable protein may be stored with little increase in body fat.

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GROWTH, VARIABILITY OF GROWTH, AND MARKET QUALITY OF BROILERS ON MASH AND PELLETS¹

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ABSTRACT

Floor-reared broilers fed a broiler ration in pellet form were heavier at all ages (6, 9, 10 and 11 weeks) than birds fed the same ration in mash form. The percentage difference in weight between mash and pellet-fed birds increased to a maximum at 11 weeks for males and at 10 weeks for females. Four different genotypes (broiler strains) all showed a growth response to pellets although there was some evidence of a differential response.

Coefficients of variation of body weight were higher at all ages for pellet-fed birds of both sexes. Females generally had higher coefficients of variation than males of comparable age on the same form of feed. For both sexes there was a slight tendency toward lower coefficients of variation with age. The data also indicated that, on both mash and pellets, separate rearing of the sexes resulted in greater variability than rearing the sexes together. There were, however, no differences in body weight due to the method of rearing the sexes. Among pellet-fed birds there was a greater incidence of breast blisters and incomplete back-feathering and the latter resulted in down-grading for dressing. Pellet-fed birds however graded higher for fat.

INTRODUCTION

It is well known that the variability of a population can be altered by nutritional and environmental factors. Zucker *et al.* (7) have made an extensive review of the literature and cite a number of investigations on both plants and animals in which this has been demonstrated.

Zucker *et al.* (7) analysed growth data on 3- and 4-week-old chicks and reported a highly significant negative correlation between rate of growth and the coefficient of variation. From the results of their own study and evidence from the literature these workers concluded that the "negative correlation between variability and rate of growth has a general validity". Becker and Berg (3) also found that the coefficient of variability was lowest for chicks on the diet which resulted in the faster growth. In their study they also found the method of rearing the sexes influenced variability of body weight with lower coefficients of variation occurring, in general, when the sexes were reared separately.

In a study involving the effect of pelleting rations on the growth of chicks, Lindblad and Aitken⁶ observed that, in addition to showing a growth response, pellet-fed birds also had a lower phenotypic variance than mash-fed birds. A growth response due to pelleting the ration has been demonstrated by a number of workers. [See literature review by Allred *et al.* (1 and 2)]. However, a review of the literature indicated that most investigators who have studied the effects of pelleting the ration on growth have not mentioned any effect on variability. Stewart and

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⁶Lindblad, G. S., and J. R. Aitken. Mash, crumbles and pellets on a high barley ration. Unpublished research. Poultry Division, Central Experimental Farm, Ottawa. 1955.

Upp (6) reported no difference in variability between pellet- and mash-fed birds but neither did these workers find any growth improvement from feeding pellets.

The feeding of the ration in pelleted form is common commercial practice. If greater uniformity in growth is achieved, then this would have important implications. Such a decrease in variability would be of particular interest and importance in the design of both nutritional and breeding studies. The main objective of this study was, therefore, to compare the variability of floor-reared broilers fed a broiler ration in mash form with broilers fed the same ration but in pellet form. Three experiments were carried out in which the main comparisons were between mash and pellet feeding. In addition, in one of these experiments four broiler strains were compared. And since the facilities permitted, in one experiment a comparison was also made between rearing the sexes separately and rearing them together in order to determine if this management practice might also affect the growth and variability of broilers.

MATERIALS AND METHODS

Three experiments were carried out in the spring and summer months of the same year (1957), but each at a different Experimental Farm of the Canada Department of Agriculture.

Chicks for all experiments were hatched at one location (Ottawa) and shipped by air or rail to the co-operating farms. There were three separate hatches, one for each experiment. Crippled chicks were culled at hatching but no culling was practised at any other time.

Standard commercial broiler rearing procedures were followed in the rearing of the birds and these were, as far as possible, uniform for the three experiments. One square foot of floor space was allowed per bird.

TABLE 1.—COMPOSITION OF RATION

Ingredients	Per 100 lb.
	lb.
Ground corn	31.5
Ground wheat	31.5
Fish meal (65%)	4.0
Meat meal (50%)	4.0
Soybean oil meal (44%)	20.0
Dehydrated cereal grass	3.0
Skim milk powder	2.0
Corn distillers' dried solubles	2.0
Steamed bonemeal	1.5
Iodized salt	0.5
D L methionine	23 gm.
Dry D ₃	37,000 I.U.
Riboflavin	1 gm.
Niacin	1 gm.
Vitamin B ₁₂	300 mg.
Manganese sulphate	4 gm.
Procaine penicillin	200 mg.
Nicarbazin	6 gm.

TABLE 2. — DESIGN OF EXPERIMENTS

	Experiment		
	Lethbridge	L'Assomption	Charlottetown
Number of strains	1	4	1
Method of rearing sexes	Separately and together	Together	Together
Number of chicks started	3072	2720	1920
Number of pens	12	8	4
Number of birds weighed per pen:			
6 weeks	All ¹	All	All
9 weeks	48(96) ²	40(80)	80(160)
10 weeks	All	All	80(160)
11 weeks	48(96)	40(80)	80(160)
Number slaughtered at			
10 weeks per pen	48(96)	40(80)	80(160)

¹ All birds alive at time of weighing² The first figure refers to the number of each sex weighed in each pen, and the figure in parentheses to the total number of both sexes weighed.

The composition of the broiler ration (22 per cent protein) is shown in Table 1. This ration was mixed by a commercial feed company with the total requirements for each experiment mixed at the same time. One-half of the mix was pelleted and the other half left in mash form. The pellets were fed in "crumble" form to 3 weeks and the change to pellets was made gradually between the third and fourth week.

Details of experimental design for each of the three experiments are shown in Table 2. In the Lethbridge experiment the sexes were reared together in four pens and separately in eight pens, four of each sex.

Chicks from the Ottawa Meat Control Strain (a synthetic strain in which the 1957 generation chicks were the progeny of a four-way cross of four dominant white meat breeds) were used in all experiments. This strain was used exclusively in the Lethbridge and Charlottetown experiments, and with three other broiler strains in the L'Assomption experiment. These other strains included N.H. x B.P.R. crossbreds, and two commercial crossbred strains of broiler chicks.

Individual body weights were obtained at 6, 9, 10 and 11 weeks of age. In each experiment only a random sample of birds was weighed at 9 and 11 weeks (and at 10 weeks in the Charlottetown experiment). The random sample was taken at 9 weeks and the same birds were again weighed at 11 weeks.

At 10 weeks, a random sample of birds was slaughtered to obtain market grading data. Official Poultry Products Inspectors of the Canada Department of Agriculture factor-graded the birds according to grade standards.

Body weight means and coefficients of variation were calculated on a "within-pen" basis. Weighted (η) values of these individual "within-pen" means and coefficients were used in combining data from more than one pen or experiment. All combined estimates of coefficients of variation were calculated as arithmetic means of the "within-pen" coefficients.

In order to equalize the η values between pens within experiments, only body weight data on birds comprising the random samples selected

at 9 and 10 weeks of age were used to obtain coefficient of variation estimates for all ages.

Statistical analyses of the body weight data were restricted to analysis of variance of body weights. Because of the doubtful validity of utilizing analysis of variance techniques for testing differences among coefficients of variation, and also due to the limitations imposed by a lack of uniform facilities for each experiment, coefficients of variation were not subjected to statistical analyses.

RESULTS AND DISCUSSION

Data for the Ottawa Meat Control Strain, combined over all experiments, are presented in Table 3. Pellet-fed birds of both sexes were heavier at all ages. On a percentage basis the trend was for the difference between the means of the pellet- and mash-fed birds to become greater with age. This was particularly true for males which had a percentage difference of 6.7 at 6 weeks and 9.6 at 11 weeks. On the other hand, females showed this trend only to 10 weeks and the percentage difference at 11 weeks was less

TABLE 3.—MEANS AND COEFFICIENTS OF VARIATION OF BODY WEIGHT OF BROILERS AT 4 AGES ON MASH AND PELLETS (COMBINED DATA FROM 3 EXPERIMENTS FOR OTTAWA MEAT CONTROL STRAIN)

Age (weeks)		Males			Females		
		Mash	Pellets	Diff. (%)	Mash	Pellets	Diff. (%)
6	Mean (gm.) ¹	691	737	6.7	591	623	5.4
	C.V. ¹	9.5	10.0		9.8	10.5	
9	Mean (gm.)	1268	1378	8.7	1036	1095	5.7
	C.V.	9.1	9.4		9.6	9.7	
10	Mean (gm.)	1442	1569	8.8	1162	1245	7.1
	C.V.	9.0	9.2		8.6	9.2	
11	Mean (gm.)	1659	1819	9.6	1322	1392	5.3
	C.V.	9.0	9.3		9.8	10.0	

¹ For each sex on each treatment means are based on an average of 1350, 392, 1060, and 392, and coefficients of variation on 984, 392, 624 and 392 individuals at 6, 9, 10 and 11 weeks of age respectively (see text and Table 2 for computational details).

TABLE 4.—MEANS AND COEFFICIENTS OF VARIATION OF 10-WEEK BODY WEIGHT OF 4 BROILER "STRAINS" ON MASH AND PELLETS (L'ASSOMPTION EXPERIMENT)

"Strain" (Code)		Males			Females		
		Mash	Pellets	Diff. (%)	Mash	Pellets	Diff. (%)
1	Mean (gm.) ¹	1430	1528	6.9	1131	1228	8.6
	C.V. ¹	9.4	9.9		9.8	8.1	
2	Mean (gm.)	1618	1664	2.8	1275	1301	2.0
	C.V.	6.0	8.2		6.0	6.7	
3	Mean (gm.)	1657	1733	4.6	1321	1349	2.1
	C.V.	8.7	8.8		7.9	8.9	
4	Mean (gm.)	1503	1616	7.5	1194	1250	4.7
	C.V.	7.9	7.8		8.2	9.6	

¹ Each mean is based on an average of 160 and each coefficient of variation on 80 individuals.

than at any earlier age. This difference between males and females is, in all probability, a reflection of the differential growth pattern of the sexes.

The three other broiler strains tested in the L'Assomption experiment also showed a growth response to pellets (Table 4). However, the percentage difference in mean 10-week body weights between pellet- and mash-fed birds varied to some extent between strains. There was no relationship between the over-all mean 10-week weight of the strain and its growth response to pellets. The heaviest strain, Strain 3, and the lightest, Strain 1, both showed a greater weight increase from pellets than Strain 2 which was intermediate in body weight.

Limitations in the design of this experiment prevented carrying out a strictly valid statistical test of the interaction between strain $\times$ form of feed. However, assuming negligible "pen effects", the F test did show this interaction to be statistically significant ($P < 0.01$). Experience with the other experiments in which it was possible to test this assumption of negligible pen effects indicated that this assumption might not be entirely tenable, primarily due to the time-interval between pen weights as a result of weighing individually a large number of birds. These data are, nevertheless, suggestive of a genotype $\times$ environment interaction and it is felt that this question might be profitably investigated further.

From the differences in magnitude of coefficients of variability as seen in Table 3 between pellet- and mash-fed birds, it is clear that the variability was influenced by the form in which the ration was fed.

In every case the coefficients of variation of pellet-fed birds were slightly higher than those for mash-fed birds of comparable age and sex. Two other trends in these coefficients of variation were also apparent. Females generally had higher coefficients than males of the same age on the same form of feed, and there was a tendency for both sexes toward slightly decreasing coefficients with age, particularly as between 6 weeks and the other ages. This trend toward lower coefficients of variation with age is in general agreement with the results of Becker and Berg (3) who found this same trend after 3 weeks for birds reared to 8 weeks of age.

There were (Table 4), as would have been expected, some differences in the magnitude of coefficients of variation among strains. Strain 2, on the average, had the lowest and Strain 1 the highest coefficients of variation. In general, both sexes of all strains had higher coefficients of variation on pellets than on mash. The two exceptions, namely, females of Strain 1 and males of Strain 4, may be the result of sampling error, as these coefficients are based on somewhat lower η values than the coefficients of variation in Table 3. Larger coefficients of variation on pellets was the consistent pattern for both sexes of Strain 1, as seen in Table 3, so it would appear reasonable to assume these reversals may be due to the larger sampling error.

The evidence from these studies indicates quite clearly that although heavier birds were obtained with pellet feeding the growth improvement was accompanied by an increase in the variability of the population. These results are in agreement with the general body of evidence with respect

to a growth response from pellets. [See Allred *et al.* (2)]. They are not, however, consistent with the findings of Lindblad and Aitken* nor the pattern of evidence in the literature indicating a negative relationship between variability and growth [e.g., Becker and Berg (3), and Zucker *et al.* (7)].

The pelleted experimental rations on which Lindblad and Aitken* found a growth response accompanied by a decrease in variability differed, particularly in grain and fibre content, from the ration used in this study. Since it has been found by Allred *et al.* (1) that response to pelleting depends in part on the composition of the ration, it is possible that disagreement between the findings reported here and those of Lindblad and Aitken might be due, in part at least, to the different rations. It is felt, however, that a somewhat more evident explanation to account for this positive relationship between growth and variability in these data might lie in the "social" environment created in the pellet-fed pens. It is well known, as has been pointed out by Heuser (4) and others, that pellet-fed birds tend to be more cannibalistic and aggressive than birds on mash. Evidence for this is provided by observations on incomplete back feathering (Table 6), made in connection with the market quality aspect of this study. Forty-two and eleven per cent of males and females respectively on pellets, as against only twelve and one per cent on mash, were classified as having some degree of incomplete back feathering. This greater aggressiveness of birds on pellets could tend to create "within-pen" variability. This increased variability due to this cause might also be expected to be greater in fairly large floor-pen units used in these experiments than in smaller floor-pen or battery-pen units.

On the assumption males would tend to be more aggressive than females, data from the Lethbridge experiment, in which the sexes were reared separately as well as together, although not disproving, do not entirely support the explanation advanced above for increased variability. As seen in Table 5, both sexes when reared separately had higher coefficients of variation than when reared together. Whereas floor space was allotted on a per bird basis, the more crowded conditions and the greater aggressive-

*See footnote page 7.

TABLE 5.—MEANS AND COEFFICIENTS OF VARIATION OF 10-WEEK BODY WEIGHT WITH SEXES REARED TOGETHER AND SEPARATELY ON MASH AND PELLETS (LETHBRIDGE EXPERIMENT)

Method of rearing sexes		Males			Females		
		Mash	Pellets	M-P	Mash	Pellets	M-P
Together	Mean (gm.) ¹	1455	1571	1513	1182	1234	1208
	C.V. ¹	8.2	8.9	8.6	7.6	8.6	8.1
Separately	Mean (gm.)	1423	1562	1493	1155	1249	1202
	C.V.	9.0	9.6	9.3	8.0	9.2	8.6

¹ For each sex on each form of feed means are based on an average of 245 and 490 individuals for the sexes reared together and separately respectively. Corresponding coefficients of variation are based in each case on 192 individuals.

ness in pens of males would appear to be a reasonable explanation for the greater variability of males reared separately; but this explanation would not hold for females reared separately. Considering both sexes, there does not appear to be any immediate explanation for these results. However, it should be pointed out that, with respect to agreement between males and females, one feature inherent in the design places some slight limitation on the interpretation. Means and coefficients of variation for males and females reared together were calculated from data on birds in the same pens and, therefore, would not be strictly independent of pens, which would be the case for data for males and females reared separately. Becker and Berg (3) found that separating the sexes resulted in lower coefficients of variation, which is not in agreement with the results of this study. However, in their study the birds were battery-reared in fairly small units which may account for these conflicting results.

The over-all mean body weights of both males and females, as seen in Table 5, differed slightly according to the method of rearing the sexes. Birds which were reared with the sexes together or intermingled in the same pens were slightly heavier than were those birds that came from pens in which the sexes were reared separately. For neither sex, however, were these differences due to rearing method found to be statistically significant, which is in agreement with the results reported by others [Smith *et al.* (5), Becker and Berg (3)].

From the market quality standpoint there were both advantages and disadvantages to the pellet feeding. Reference was made previously to the much greater number of pellet-fed birds than mash-fed birds classified as having incomplete back feathering. Also, as seen in Table 6, the incidence of breast blisters was greater among males on pellets, but there were no differences between mash- and pellet-fed birds in the percentage of crooked

TABLE 6.—EFFECT OF FORM OF FEED ON CARCASS DEFECTS AND MARKET GRADING¹

	Males		Females	
	Mash	Pellets	Mash	Pellets
<i>Defects</i>				
Incomplete feathering (%)	12	42	1	11
Crooked keels (%)	4	3	2	2
Breast blisters (%)	3	15	1	1
<i>Market Grading</i> ²				
Conformation 1 (%)	97	97	99	99
2 (%)	3	3	1	1
Fleshing 1 (%)	88	93	97	98
2 (%)	12	7	3	2
Fat 1 (%)	27	53	47	61
2 (%)	73	47	53	39
Dressing 1 (%)	92	77	91	92
2 (%)	8	23	9	8

¹ Combined data from three experiments for Ottawa Meat Control Strain. 392 individuals of each sex on each treatment classified and graded.

² 1 and 2 refer to grade class; all birds graded in two top C.D.A. grade classes, i.e. Sp. (*Special*) and A are included in Grade Class 1, and B's or lower (C and D) are included in Grade Class 2.

keels. The higher incidence of blisters among pellet-fed birds is in all probability due to the greater weight of these birds.

These differences in feathering between pellet- and mash-fed birds were reflected in the market grades for the dressing grade factor for males but not for females. Twenty-three per cent of the males on pellets were graded B or lower compared to only eight per cent of the males on mash. Contrary to expectation, the higher incidence of breast blisters among pellet-fed birds did not result in lower grades for the conformation grade factor. No differences were observed between mash- and pellet-fed birds for fleshing grades, but the pellet-fed birds were graded markedly higher for fat than the mash-fed birds.

This study would indicate, therefore, that the most serious disadvantage of pellets from the market quality standpoint appears to be incomplete feathering which resulted in down-grading for dressing. This incomplete feathering presumably arises from the feather-picking habit, and it would be expected that this disadvantage could be overcome at least to some extent by invoking management practices such as de-beaking.

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EFFECT OF ESTRADIOL AND TESTOSTERONE INJECTIONS AND THYROIDECTOMY ON WOOL GROWTH IN SHEARLING SHEEP¹

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ABSTRACT

An experiment was conducted to determine the effect of relatively high doses of estradiol and testosterone, with and without thyroidectomy, on clean wool production and certain endocrine glands of shearling sheep. The administration of 5 milligrams of estradiol intramuscularly at bi-weekly intervals to sheep with thyroids caused a reduction in the average weight of clean wool ($p < .05$) and fibre length ($p < .01$). This treatment produced increases ($p < .01$) in thyroid, pituitary, and adrenal weights. The administration of estradiol to thyroidectomized ewes reduced the weight of clean wool ($p < .05$) when compared with the thyroidectomized controls but no such response was observed in wethers. Testosterone administered at levels of 175 milligrams bi-weekly produced no effect on weights of clean wool, in ewes or wethers, but when it was administered to thyroidectomized animals average fibre lengths were decreased ($p < .05$).

INTRODUCTION

Previous studies (12) at the Research Station, Lethbridge, Alberta, concerning the effect of the administration of certain sex hormones at high therapeutic levels on wool growth in sheep have indicated that estradiol injected intramuscularly caused a reduction ($p < .01$) in the average weight of clean wool obtained from measured skin areas. This treatment also reduced average thyroid weight but increased pituitary and adrenal weights. It was suggested that the reduction in wool production resulted from a lowered thyroid activity and from adrenal cortical stimulation. Injections of testosterone increased ($p < .05$) the weight of clean wool when the sheep were treated with high doses (up to 175 milligrams) during the first part of the experiment and lower doses (75 milligrams) during the latter part. In a subsequent experiment (12), where 125 milligrams per head were injected bi-weekly throughout the trial, no such response was obtained with testosterone. In the latter experiment thyroid weights were increased.

The present study was undertaken to determine the effect on wool growth of 1) high levels of estradiol and testosterone, and 2) thyroidectomy.

MATERIALS AND METHODS

Forty-eight grade Corriedale shearling sheep, half of which were ewes and half wethers, were used in this study. They were divided at random into three equal groups on the basis of wool production and body weight. Ewes and wethers were distributed equally and a further division was made by thyroidectomizing one-half of the ewes and wethers in each group 5 weeks before treatment was begun. Despite the fact that great care was taken in the extirpation of the thyroid gland to ensure complete removal (the blood-vessels at each end of the gland were ligated), some thyroid regrowth occurred in 16 out of 24 animals. Histological examination of the regrowth tissue showed that it was normal thyroid. In each case, one pair of parathyroid glands was removed with the thyroids. No sham operations were performed on the controls.

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TABLE 1.—AVERAGE WEIGHTS OF CLEAN WOOL FROM TATTOOED AREAS AS AFFECTED BY THE ADMINISTRATION OF TWO SEX HORMONES AND THYROIDECTOMY

Collection period ending (1957)	T R E A T M E N T							
	Estradiol		Testosterone		Control		Period average	
	With thyroids	Thyroid- ectomized	With thyroids	Thyroid- ectomized	With thyroids	Thyroid- ectomized	With thyroids	Thyroid- ectomized
Weights of clean wool (mg.)								
Ewes								
June 19 (preliminary)	87.9	71.8	89.0	71.2	87.6	70.0	88.2	71.0
July 18	80.8	59.2	89.5	72.6	101.0	80.9	90.4	70.9
August 14	70.5	39.1	87.0	59.6	87.8	70.8	81.8	56.5
September 11	67.8	43.6	79.0	58.8	87.5	70.2	78.1	57.5
October 9	65.8	47.2	82.3	55.1	76.4	65.2	74.8	55.8
November 6	65.9	53.2	76.1	51.0	69.2	60.1	70.4	54.8
December 4	58.5	48.9	72.6	44.1	66.2	51.7	65.8	48.2
Lot averages	68.2 ¹	48.5 ²	81.1	56.9 ¹	81.4	66.5 ¹	76.9	57.3
Wethers								
June 19 (preliminary)	86.2	71.7	89.3	72.6	86.7	72.0	87.4	72.1
July 18	75.8	78.8	90.1	64.2	100.5	66.0	88.8	69.7
August 14	60.3	67.6	87.7	62.8	88.0	60.3	78.7	63.6
September 11	58.5	67.1	81.9	61.8	90.2	64.7	76.7	64.5
October 9	53.7	66.9	72.3	57.6	81.6	59.1	69.2	61.5
November 6	51.6	65.4	68.1	52.2	78.6	66.8	66.1	61.5
December 4	48.3	55.5	67.1	49.3	76.5	62.8	64.0	55.9
Lot averages	58.0 ¹	66.9	77.9	58.0 ¹	85.9	63.3 ¹	73.9	62.7

¹Significantly lower than the controls with thyroids ($p < .05$)²Significantly lower than the thyroidectomized controls and estradiol group with thyroids ($p < .05$)

In the present studies high levels of hormone treatments were used. Unpublished data* from this Station on the effect of 1, 2, 3, 4, 5, and 6 milligrams of estradiol intramuscularly at 14-day intervals have indicated that 1, 2, and 6 milligrams produced no change in wool growth when compared with controls, whereas 3, 4, and 5 milligrams reduced wool growth ($p < .05$). The reductions in growth caused by injections of both 4 and 5 milligrams of estradiol were of equal magnitude, but were greater than that obtained from an injection of 3 milligrams. On this basis it was decided to use 5 milligrams of estradiol per head at 14-day intervals.

In the case of testosterone (12) it has been found that varying doses of up to 175 milligrams produced an increase in wool growth ($p < .01$) while regular doses of 125 milligrams gave no response in growth. Consequently, it was decided to use 175 milligrams at 14-day intervals throughout the trial.

Lot 1—2.5 cubic centimetres of estradiol cyclopentylpropionate in cottonseed oil (5 milligrams ECP)

Lot 2—7 cubic centimetres of testosterone in propylene glycol, alcohol, and redistilled water (175 milligrams testosterone)

Lot 3—5 cubic centimetres of physiological saline.

Feeding of the sheep began on May 24, injections commenced on June 21, and the animals were slaughtered after final weighing on December 4, 1957. The animals were fed individually, the feed intake being the same for all sheep to reduce individual variation in wool growth. The average daily consumption was 1.5 pounds of chopped alfalfa hay and 1.0 pound of whole oats, with deviations between lots of not more than .01 pound. A mineral mix of bone meal, salt, and trace minerals was fed *ad lib*. The calculated daily intakes of digestible crude protein and total digestible nutrients, based on Morrison (9), were 0.25 and 1.5 pounds, respectively. The average daily gains of all lots ranged from .19 to .24 pounds and indicate that, though feed intake was somewhat restricted below the N.R.C. standards (11), the gains were satisfactory. The effects of hormone injections and thyroidectomy on liveweight change have been discussed by MacDonald and Slen (7).

The thyroid, pituitary, and adrenal glands were removed and the warm weights taken immediately after slaughter. Wool production data were obtained by clipping samples at 28-day intervals from the same measured areas (2 x 2 centimetres) tattooed on the right shoulder of each sheep. The samples were analysed for clean weight, fibre length, and fibre thickness to study periodic variation and to obtain total production. The hormone treatments were continued for six consecutive wool-collection periods totalling 168 days.

The data were analysed on the basis of total weight of clean wool and length of fibre produced during the experimental period, and average fibre thickness and number of fibres per unit of skin area (density) for all periods. Analysis of covariance was used for the latter two characteristics to adjust for initial differences.

*The effect of varying levels of estradiol on weight of clean wool. Wool Research Laboratory, Lethbridge, Alta. 1958.

RESULTS

Weight of Clean Wool

The average weights of clean wool taken at six different collection periods from tattooed areas at 28-day intervals and the over-all averages are shown in Table 1. The periodic data have been included to show that, where responses from the hormone were obtained, they occurred almost immediately and were sustained for the duration of the trial.

Estradiol given intramuscularly at 14-day intervals reduced the average weights of clean wool ($p < .05$) from intact ewes and wethers by approximately 25 per cent when compared with similar controls. In the case of thyroidectomized ewes estradiol further reduced ($p < .05$) the weight of clean wool by 29 per cent below that of the thyroidectomized controls. However, when thyroidectomized wethers were treated with this hormone, no change occurred in wool production nor was there any sex $\times$ treatment interaction. It is interesting to note that the estradiol treatment of both normal ewes and wethers and thyroidectomy *per se* produced the same reduction in average weights of clean wool when compared with normal controls. Thyroidectomy caused an over-all reduction in wool production of approximately 20 per cent despite the fact that some regrowth of thyroid tissue occurred in several animals.

The use of testosterone on both intact and thyroidectomized wethers had no effect on wool growth when compared with the appropriate controls.

Fibre Characters

The average fibre lengths, thicknesses, and densities are shown in Table 2.

The average fibre lengths obtained from estradiol-treated ewes and wethers with thyroids were reduced ($p < .05$) when compared with normal controls. However, the estradiol treatment of thyroidectomized sheep caused no further decrease in fibre length when compared with thyroidectomized controls, as was obtained in weights of clean wool. On treatment with testosterone no response in the lengths occurred in intact wethers but there was an increase ($p < .05$) in the ewes. However, there was no significant sex $\times$ treatment interaction. Fibre length decreases ($p < .05$) in both thyroidectomized ewes and wethers were obtained from testosterone treatment.

Gland Weights

The average warm weights of the thyroid, pituitary, and adrenal glands and body weights are presented in Table 3. No difference in the average weights of thyroid glands as a result of testosterone treatment was found between the intact ewes and wethers. However, an increase ($p < .01$) in thyroid weight was observed in both ewes and wethers on estradiol treatment. This is not in agreement with previous results (12), although the dosage levels were different.

TABLE 2. — FIBRE CHARACTERISTICS OF WOOL AS AFFECTED BY THE ADMINISTRATION OF TWO SEX HORMONES

Fibre characters	T R E A T M E N T							
	Estradiol		Testosterone		Control		Period average	
	With thyroids	Thyroid- ectomized	With thyroids	Thyroid- ectomized	With thyroids	Thyroid- ectomized	With thyroids	Thyroid- ectomized
Ewes								
Fibre length (mm.)	10.6 ¹	10.4 ¹	12.1 ¹	9.1 ¹	11.3	10.1 ¹	11.3	9.8
Fibre thickness (μ)	24.6	22.4	25.5	21.6	23.9	23.9	24.7	22.6
Fibre density ²	17.6	15.3	17.7	19.4	20.0	18.9	18.4	17.9
Wethers								
Fibre length (mm.)	10.0 ¹	9.6 ¹	10.4	9.1 ¹	11.1	9.8 ¹	10.5	9.5
Fibre thickness (μ)	21.2	24.3	24.0	22.5	23.8	22.2	23.0	23.0
Fibre density ²	21.6	19.2	20.2	19.8	23.4	19.6	21.7	19.5

¹Significantly different from the controls with thyroids ($p < .01$)²Number of fibres per square inch (000's)

TABLE 3. — AVERAGE GLAND AND BODY WEIGHTS AS AFFECTED BY THE ADMINISTRATION OF TWO SEX HORMONES

	T R E A T M E N T					
	Estradiol			Testosterone		Control
	With thyroids	Thyroid- ectomized	With thyroids	Thyroid- ectomized		
Ewes						
Gland weights (gm.)						
Thyroid	6.45 ¹	1.06*	4.78	2.46*	4.48	0.33*
Pituitary	1.00 ¹	1.08 ¹	0.63 ¹	0.54 ¹	0.38	0.34
Adrenal	3.44 ¹	3.20	2.50	2.61	2.50	2.85
Body weights (lb.)						
Initial	63.5	64.5	67.3	63.0	62.8	62.5
Final	110.8	107.3	104.3	102.5	106.8	102.5
Wethers						
Gland weights (gm.)						
Thyroid	5.92 ¹	1.53*	5.32	1.38*	4.27	1.15*
Pituitary	1.53 ¹	0.98 ¹	0.65	0.66	0.64	0.48
Adrenal	4.14 ¹	3.34	2.66	2.40	2.51	2.61
Body weights (lb.)						
Initial	68.5	67.8	63.0	66.3	70.0	67.8
Final	112.0	110.3	106.0	106.3	107.3	107.8

*Gland not entirely removed and some regrowth occurred
¹Significantly greater than their respective controls ($p < .01$)

Estradiol treatment caused an increase ($p < .01$) in the average weights of pituitary glands in both ewes and wethers when compared with the controls irrespective of whether the animals were thyroidectomized. The use of testosterone had no effect on the pituitaries of normal or thyroidectomized wethers but resulted in an increase ($p < .05$) in normal and thyroidectomized ewes.

An increase ($p < .01$) in the average adrenal weights was observed in both ewes and wethers treated with estradiol when compared with normal controls but had no effect on thyroidectomized animals. Testosterone treatment produced no response in adrenal weights in any of the experimental groups. Considering the controls only, it was found that thyroidectomy *per se* produced no changes in the average weights of either the pituitary or adrenal glands.

DISCUSSION

Recent research has shown that certain hormones have an important influence on the growth of wool (3, 4, 5, 6), although it is evident that the relationship is complex. The secretions of the anterior pituitary are essential for normal wool production, as indicated by hypophysectomy which will lead to a severe depression of wool growth (3). Injected pituitary growth hormone stimulated wool growth in intact sheep but failed to restore wool growth in hypophysectomized animals. This suggested that the growth hormone was not the only pituitary secretion necessary for normal wool growth (4). It also was noted that the injection of either ACTH or the hormones of the adrenal cortex depressed the growth of wool (6). The hormones produced by the adrenal cortex appeared to have a direct action on the wool follicle since the topical application of an ointment containing a cortisone analogue to a restricted area of the skin suppressed wool growth, but only on the treated area (6). Thyroid involvement in the wool growth complex has also been demonstrated in that thyroidectomy depressed and thyroxine injection stimulated the growth of wool (5, 8). The effects of the former treatment have been confirmed in the present experiments.

It is well known that the parenteral or oral administration of estrogenic substances to both cattle (10) and sheep (1) stimulate body growth. Although the physiological mechanisms by which this stimulation is achieved are not fully understood, the practical implications of the phenomenon are obvious. Wool is an important by-product of lamb production and it was of considerable practical as well as fundamental interest to know what effect the injection of the sex hormones have on the growth of wool. The present results and those reported in a previous communication (12) show that the intramuscular injection of estradiol to sheep depressed the growth of wool when compared with non-treated controls. In addition, it would appear that, in the ewe, the effect of the estradiol was independent of the activity of the thyroid gland. Thyroidectomy and estradiol administration reduced wool growth by amounts of about equal magnitude but the effects of the two treatments were additive when applied simultaneously to the same animal. Similar results were not forthcoming with the wethers

which may point to differences in the endocrine control of wool growth between wether and ewe. Testosterone injection evoked no wool growth response in either ewes or wethers and did not alter the typical response to thyroidectomy.

It cannot be ascertained from these experiments whether the inhibition of wool growth following estradiol injection was due to a direct action of the hormone on the wool follicle or whether the effect was due to the involvement of other endocrine organs. There is evidence, however, which might suggest that the action of the estradiol was mediated through the pituitary-adrenal axis. From their experiments on the implantation of lambs with diethylstilbestrol, Clegg and Cole (2) suggested that this compound gave rise to increased adrenal cortical activity as judged by a resulting depression of the blood eosinophil count. Linder and Ferguson (6) found that the number of circulating eosinophils was reduced by treatment with adrenocorticotrophic hormone. A complex endocrine involvement also may be inferred from the fact that estradiol treatment of both the ewes and the wethers in the present experiments led to significant increases in the weights of the thyroid, adrenal, and pituitary glands. Further work is under way to delineate the response of the wool follicle to the injection of female sex hormone.

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THE EFFECT OF FINE GRINDING OF HAY ON RATION DIGESTIBILITY, RATE OF PASSAGE, AND FAT CONTENT OF MILK¹

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ABSTRACT

Three series of digestion trials, involving a total of 20 lactating cows, were conducted to compare the digestibility and rate of passage of hay ground to various degrees of fineness, with that of unground hay.

Grinding the hay in a ration composed of two parts hay and one part concentrate by weight produced an earlier initial excretion of hay residues in all cows. Excretion of finely ground hay was also more rapid than unground hay throughout the period. The change in excretion time due to grinding was statistically significant.

The most marked effect on digestibility was the highly significant decline ($P < 0.01$) in digestibility of fibre and/or cellulose as a result of grinding hay. This was mainly responsible for the highly significant decrease ($P < 0.01$) in dry matter digestion. Grinding also significantly lowered ($P < 0.05$) the digestibility of ether extract in trials of series II and of protein and N.F.E. in trials of series III.

A relationship was established between rate of passage of hay and digestibility of the total ration. The finer the hay was ground, the greater was the depression in digestibility of the total ration and the faster the rate of excretion. The large depression in the digestibility of the cell wall constituents was associated with a marked decrease in milk fat percentage.

INTRODUCTION

During the past 20 years, research has shown that restricting hay intake of liberally fed cows to less than 8 pounds per day had a depressing effect on milk fat percentage (Balch *et al.*, 3, 4; Ensor *et al.*, 9; Loosli *et al.*, 13; Powell, 20; Tyznik, 22; Van Soest, 23). Depression of fat percentage also resulted when finely ground hay was liberally fed as the sole roughage (Ensor *et al.*, 9; Van Soest, 23).

The effect of grinding hay on digestibility of a ration is somewhat controversial. Some researchers (Balch, 2; Bechdel *et al.*, 5, 6; Blaxter and Graham, 7; Forbes *et al.*, 11; Heller *et al.*, 12) have reported that grinding the hay in the ration caused a decrease in digestibility, especially of the cell wall constituents, while others (Meyer *et al.*, 15; Morrow and La Master, 16; Olson, 18) have found no significant differences in digestibility between ground and/or pelleted and unground hay. In most cases, failure to report the degree of fineness of grinding in absolute values makes it difficult to interpret the results. The level of intake may also account for some of the differences noted.

Few reports have appeared in the literature concerning the digestibility of finely ground roughage by lactating cows. This might be an important factor in the depressing effect on milk fat percentage which is known to occur in cows fed finely ground roughage (9, 23). This experiment was conducted to study this question and to relate digestibility to the rate of passage of hay through the digestive tract.

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TABLE 1 — COWS USED AND MEAN MILK FAT PERCENTAGES DURING THE DIGESTION TRIALS
(Letter following cow number indicates breed)

Cow number	Age of cow (years)	Mean weight of cow during experiment (lb.)	Stage of lactation (months)	Mean milk fat percentage during trials on	
				Ground hay	Long hay
Series I					
347-H	5	1425	8	2.90	3.32
253-H	6½	1310	13	2.86	3.26
49-G	7	1205	10	5.34	5.58
46-G	7	1000	6	4.30	4.44
19-G	9½	840	6	3.70	3.98
31-A	3½	1140	12	4.50	4.95
116-G	4	768	4	3.76	3.96
68-G	6½	890	5	3.57	3.82
Series II					
224-H	7½	1500	7	2.08	3.25
411-H	5	1310	6	2.90	3.00
19-G	10	897	13	4.02	4.81
49-G	8	1064	6	4.70	4.91
46-G	8	1000	4	3.96	4.03
31-A	4	1110	4	3.53	3.94
187-H	8	1233	7	2.45	3.17
33-A	3½	1020	2	3.73	4.00
Series III					
46-G	8	995	6	3.98	4.23
31-A	4	1120	6	3.69	4.19
187-H	8	1240	9	2.76	3.58
33-A	3½	1030	4	3.49	4.40

MATERIALS AND METHODS

Plan of Experiment

An experiment (beginning October, 1954, and ending February, 1956), involving three series of digestion trials, was conducted with lactating dairy cows in the experiment station herd (Table I). The hay* was ground to a different degree of fineness for each series (Table 2) by using a 1/16-inch screen in a hammer mill operated at a different speed for each of the three series. A 2-cow digestion stall, especially designed for balance trials, was used throughout the experiment. The daily ration fed to each cow was composed of 2 parts hay and 1 part concentrate (98 per cent corn, 1 per cent salt, 1 per cent bone meal) by weight. The cows were fed twice daily at a level of 2.5 to 3 pounds of total feed (air-dry basis) per 100 pounds body weight.

Experimental ground hay was fed after the cows were depressed in milk fat by feeding a ration of 2 parts commercial alfalfa meal and 1 part ground concentrate, since the intent was that the digestibility of the ground hay be determined under conditions producing a depression of milk fat. The ground hay to be tested was then substituted for the commercial alfalfa meal for a preliminary period of at least 10 days.

*In Series I, hay was a mixture of equal parts alfalfa and grasses of fair quality. In Series II and III, it was second-cutting alfalfa-grass mixture of good quality with alfalfa predominating.

TABLE 2. — DISTRIBUTION OF PARTICLE SIZE OF GROUND HAY USED IN THE DIGESTION TRIALS AS DETERMINED BY U.S. STANDARD SIEVES

Sieve No.	Sieve opening	Per cent retained		
		Series I (Coarse grind)	Series II (Med. fine grind)	Series III (Very fine grind)
20	0.84 mm.	8.0	6.0	0.0
40	0.42 mm.	32.0	29.0	19.0
60	0.250 mm.	27.0	24.0	21.0
80	0.177 mm.	16.0	17.0	20.0
100	0.149 mm.	5.0	6.0	9.0
Not retained	flour	12.0	18.0	31.0

Digestibility Trials

Following this preliminary period, the pair of cows was transferred from stanchions to the digestion stalls. Three days were allowed to permit them to adjust to their new environment before starting the 10-day total collection period on ground hay. At the end of this first collection period, the cows were shifted to long hay (from the same lot as the ground hay) for a transition period of 6 days. Then a collection period of 10 days on long hay followed. Ground hay feeding always preceded the long hay period. This order was required rather than alternating, because of the relatively long and irregular interval for the fat-depressing effect of the ground hay to appear, as contrasted to the quick recovery when shifted from ground to long hay.

Feeds and feces were analysed for nitrogen, ether extract, crude fibre, N.F.E.* and ash according to A.O.A.C. procedures (1). Crude cellulose was determined by using a slight modification of the method of Norman and Jenkins (17), the sample first being extracted with an azeotropic mixture of ethanol-benzene (2 : 1 by volume). At the end of the procedure the cellulose content was determined by loss of weight on ashing in a muffle furnace at 600°C. for 30 minutes. Fat content of the milk was determined on 2-day composite samples by the Babcock test.

Rate of Passage of Feed

The measurement of the rate of passage of hay was made during the 10-day collection periods by using a dye method developed by Balch (2). Balch (2), working with fistulated cows, has shown that the time required for excretion of 97 per cent of the residues coming from stained ground hay introduced into the omasum was the same as the time required for excretion of 5 per cent of the undigested residues from hay (ground and/or unground), stained differently, and fed simultaneously. This "5 per cent excretion time" of food given orally was interpreted by Balch as being indicative of time taken by food residues to pass through omasum, abomasum, and intestine. After this point is reached, the rate of excretion becomes more rapid until 80 per cent of the food residues have been excreted and then diminishes rapidly. The difference in time for 80 per

*Throughout this paper, nitrogen-free-extract will be abbreviated as "N.F.E."

cent and 5 per cent of the residues to be excreted (called the "80-5 per cent excretion time") was interpreted by Balch to be indicative of the time the feed remains in the reticulo-rumen. These indices worked out by Balch (2) will be used in reporting and discussing the results obtained on the rate of passage of hay in this study.

Statistical Analysis

A "t" test of analysis was done on paired variates (each cow constituting a pair by serving as her own control) for the test of significance at the 5 per cent and 1 per cent levels of probability.

RESULTS AND DISCUSSION

It is shown in Table 3 that the digestibility of the crude fibre and/or cellulose was, in all series, lower ($P < 0.01$) in periods during which the ground hay was fed than in those in which long hay was fed. The decrease in digestibility of the cell wall constituents was greater in Series II than in Series I and greater in Series III than in Series II, possibly due to finer grinding. The difference between the values for both digestible dry matter

TABLE 3. — DIGESTIBILITY COEFFICIENTS, TOTAL DIGESTIBLE NUTRIENTS, AND MILK FAT CONTENT FOR GROUND AS COMPARED TO LONG HAY IN THE TOTAL RATION

Trials		Dry matter	Crude protein	Ether extract	N.F.E.	Crude fibre	Cellulose	T.D.N.	Fat content of milk
		%	%	%	%	%	%	%	%
8 COWS	Coarse Ground hay ^a								
	Mean	60.9	63.3	51.4	75.4	34.1	—	61.5	3.87
	s.d.	±2.0	±2.0	±5.1	±1.4	±5.8	—	±2.2	±0.8
	Long hay ^b								
	Mean	62.8	62.2	51.8	76.5	42.0	—	63.2	4.16
8 COWS	s.d.	±1.6	±1.5	±4.6	±1.4	±4.7	—	±1.5	±0.8
	$\bar{d}^1$	1.9**	-1.1	0.4	1.1	7.9**	—	1.7*	0.29
	Medium fine Ground hay ^c								
	Mean	61.4	68.0	60.9	75.6	30.8	39.4	61.6	3.42
	s.d.	±2.1	±2.4	±4.2	±1.9	±4.7	±3.7	±2.4	±0.8
8 COWS	Long hay ^d								
	Mean	65.9	68.0	64.6	77.1	46.3	52.6	66.7	3.89
	s.d.	±1.0	±2.0	±4.0	±1.3	±2.8	±3.8	±1.1	±0.7
	$\bar{d}^2$	4.5**	0.0	3.7*	1.5	15.5**	13.2**	5.1**	0.47*
4 COWS	Very fine Ground hay ^e								
	Mean	56.3	62.6	63.7	71.8	22.2	30.1	58.2	3.48
	s.d.	±0.8	±1.5	±3.9	±1.5	±3.7	±3.4	±0.8	±0.7
	Long hay ^f								
	Mean	65.6	68.4	66.5	77.4	44.4	52.8	67.1	4.10
4 COWS	s.d.	±0.8	±2.0	±5.0	±1.6	±2.0	±4.4	±0.7	±0.5
	$\bar{d}^3$	9.3**	5.8*	2.8	5.6*	22.2**	22.8**	8.9**	0.62

$\bar{d}^1$ Difference between two means (b-a)

$\bar{d}^2$ Difference between two means (d-c)

$\bar{d}^3$ Difference between two means (f-e)

* Significant at the 5% level

** Significant at the 1% level

s.d. Standard deviation

TABLE 4. — COMPARATIVE RATES OF EXCRETION OF HAY RESIDUES BY COWS FED LONG HAY AND GROUND HAY DIETS

Trials	Time required for excretion of different proportions (%) of total undigested residues from stained hay					
	Long hay diet (a)		Ground hay diet (b)		Change due grinding (a) — (b)	
	5% ¹ hours	80-5% ² hours	5% ¹ hours	80-5% ² hours	5% ¹ hours	80-5% ² hours
Series I (Coarse grind) Mean (8 cows) s.d.	21 ±3	47 ±6	16 ±2	39 ±5	5*	8*
Series II (Med. fine grind) Mean (8 cows) s.d.	22 ±2	57 ±8	16 ±2	44 ±8	6**	13*
Series III (Very fine grind) Mean (4 cows) s.d.	21.5 ±1.9	57.2 ±4.3	13 ±0.8	27 ±4	8.5**	30.2**

¹5% Indicative of time taken by hay ingesta to pass through omasum, abomasum and intestine (Balch, 2)
²80-5% Indicative of time hay ingesta remains in the reticulo-rumen (Balch, 2)

* Significant at the 5% level

** Significant at the 1% level

s.d. Standard deviation

and T.D.N. for ground vs. long hay periods, significant at the 1 per cent level in all cases but one, are mainly attributable to decreased digestibility of the crude fibre and/or cellulose.

In contrast to digestion trial results in Series I and II, the feeding of a very fine meal ration (Series III) caused a significant depression ($P < 0.05$) in digestibility of protein and N.F.E. This may be explained by either a reduced total microbial digestion occasioned by a faster removal of ingesta from the reticulo-rumen (Table 4), or the reduced breakdown of fibre which hindered the complete action of the animal's digestive enzymes. The significant decrease ($P < 0.05$) of 3.7 percentage units in ether extract digestibility noted in the ground hay trials of Series II can also be partly explained in this way.

These results confirm changes in digestibility associated with the feeding of ground hay as reported by Balch (2), Bechdel *et al.* (5, 6), Forbes *et al.* (11), Heller *et al.* (12), and Blaxter and Graham (7). The data reported by Meyer *et al.* (15), Morrow and La Master (16) and Olson (18) cannot directly be compared with the ones reported here because, on one hand, concentrate was not included in the ration and the level of intake was not the same and, on the other hand, the fineness of grinding was different.

Fat Content of the Milk

As is shown in Table 1, milk fat percentages were in all cases lower during the ground hay than during the long hay periods. These differences

were significant ($P < 0.05$) only in Series II (Table 3). The mean difference ($\bar{d}$) of 0.62 percentage unit in Series III of digestive trials was not statistically significant, probably due to the small number of cows involved in the comparison. These results confirm those of Powell (20), Porter *et al.* (19), Tyznik (22), Van Soest (23), and Ensor *et al.* (9). Evidence has been presented by Van Soest (23), Maki (14), and Ensor *et al.* (9) that the two different types of rations (low hay-high concentrates; high level of finely ground hay), which depress the fat content of the milk, produce about the same departure (less acetate, more propionate) from normal volatile fatty acid production in the rumen.

Rate of Passage of Hay

The comparative rates of excretion of ground hay and long hay are given in Table 4. An earlier initial excretion of the ground hay residues was observed in every cow, and a higher rate of excretion continued throughout the major part of the excretion period. This faster excretion of ground hay residues, as compared to long hay residues, was more pronounced in trials of Series III, presumably due to finer grinding. The change due to grinding for the 80-5 per cent excretion time (used as an index of time in the reticulo-rumen) was significant ($P < 0.05$) in Series I and II and highly significant ($P < 0.01$) in Series III. These results on the rate of passage are in agreement with the findings of Ewing and Smith (10), Columbus (8), Blaxter and Graham (7), Meyer *et al.* (15) but not with those of Balch (2). It is possible that the ground hay used by Balch (2) was coarse enough to closely follow the pattern of excretion noted for long hay or that the level of intake of ground hay was low.

Relation between Rate of Passage and Digestibility of Feed and Fat Content of Milk

The results of the three series of digestion trials confirm the observation of Balch (2) that the inclusion of ground hay in the total ration lowers markedly the digestibility of the fibrous carbohydrates. Measurements of the rate of passage of hay showed that changes in this factor were associated with changes in digestibility of the feed, particularly the cell wall constituents. Fine grinding of hay was associated with a greater depression in digestibility of the total ration and a higher rate of excretion. Balch (2) did not observe a similar association, possibly because of the lower total feed intake of his animals. Further, direct comparisons cannot be made with his data because the fineness of hay grinding is not reported in standard values.

Since the cellulolytic micro-organisms predominating in the rumen seldom survive beyond the rumeno-reticular portion of the digestive tract, it is plausible to conclude that the very marked decline in fibre and/or cellulose digestion, as a result of grinding hay, was probably due to a faster removal of food from the reticulo-rumen, as was calculated by the "80-5 per cent" excretion time. While a definite causal relationship cannot be proven from these data, it would appear that the decline in digestibility of the cell wall constituents brought about a condition in the cow marked by a decrease in milk fat per cent.

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FREE-CHOICE FEEDING OF TROLENE FOR REDUCTION OF CATTLE WARBLE INFESTATIONS (OESTRIDAE: DIPTERA)¹

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ABSTRACT

The systemic insecticide Trolene reduced the numbers of cattle grubs by 83 to 95 per cent when total doses of 180 and 250 mg. per kg. of animal weight were fed to calves in supplementary feed at daily doses of 10 and 15 mg. per kg. These reductions were significantly greater than those produced by lower daily or total doses including Trolene boluses at 105 mg. per kg. However, very high grub infestations survived in individual animals in all test groups except the two highest dosage groups. This is attributed to variation in feeding behaviour that resulted in the slower feeding animals receiving low doses of insecticide. This factor is apparently an inherent flaw in treatments based on free-choice feeding.

Grubs were not eradicated by any of the test treatments and only the 250 mg. per kg. total dosage reduced them below the level of infestation annually observed in two semi-isolated, untreated herds. Studies of the significance of this level of survival in the perpetuation of the treated infestations are necessary.

Transitory toxicity-symptoms were observed in the bolus-treated calves but not in the feed-treated calves.

INTRODUCTION

Rich and Ireland (7) previously discussed the problems associated with treatment of cattle herds on ranches in the interior of British Columbia with systemic insecticides for control of the cattle warbles, *Hypoderma bovis* (L.) and *H. lineatum* (De Vill.). The economics of ranch management frequently require that cattle remain dispersed on the ranges until early or mid-winter; thus in many instances treatment for lice and warbles cannot be applied until the weather is so cold that administration of the insecticide by mouth is the only practical method. In tests commenced in 1956 it was found that under these conditions the reductions in the number of grubs produced by Trolene³ bolus treatment were comparable to those produced in areas where early treatment is possible.

In 1957 a Trolene formulation for addition to supplementary cattle feed was introduced and received widespread testing. Knapp *et al.* (4) fed a single dose of 110 milligrams active insecticide per kilogram of animal weight to 80 calves and reported a reduction of 92 per cent in the number of grubs.

De Foliart *et al.* (1) fed 55 milligrams per kilogram daily for 2 days to 160 calves and reported reductions of from 61 to 82 per cent in grub numbers. Weintraub *et al.* (9) fed 10 milligrams per kilogram daily for 18 days to 30 calves and reported that the reduction of 94 per cent in grub members was statistically comparable to the reduction produced in a similar group by treatment with Trolene boluses at 110 milligrams per kilogram. French *et al.* (2) fed 35 milligrams per kilogram daily for 6 days to 28 steers and reported a reduction of 97.8 in grub numbers. Rich and Ireland (7) fed 10 milligrams per kilogram daily for 25 days to 30 calves and reported a reduction of 99 per cent in grub numbers that was significantly greater

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³Dow Chemical Company trade-name for veterinary insecticide formulations containing O, O-dimethyl O-(2, 4, 5-trichlorophenyl) phosphorothioate, formerly known as "Dow ET57". The chemical is now known in the United States by the common name of *ronnel*.

than the 83 per cent reduction produced in a similar group by Trolene bolus treatment at 105 milligrams per kilogram. Norris and Howe (6) reported high reductions in grub numbers in widespread tests and suggested additional research to establish reliable dosage information.

A study was carried out in 1957-1958 of the relationship of daily and total feeding rates of Trolene to reductions in the number of grubs and is discussed herein.

MATERIALS AND METHODS

Test Animals

The test animals were grade Hereford calves born prior to June 1, 1958, from the herd of the Harper Ranch, Kamloops, British Columbia. They had ranged as a group from birth and thus were uniformly exposed to warble infestation. Medium-sized calves were selected to give a high degree of uniformity of weight and vigour. Selection was made on December 10, 1958, the day after the calves had been separated from the cows for weaning.

Eight test groups, each of 30 calves, were selected, weighed and marked with numbered ear-tags in accordance with a previously prepared randomization schedule. Subsequent sale of some of the calves to calf-club groups reduced the final data on several groups to less than 30 calves.

Insecticide Formulations

The insecticide formulation added to supplement feed consisted of fine cottonseed meal impregnated with 10 per cent by weight of active insecticide. The boluses were the standard commercial form containing 40 per cent by weight of active insecticide.

Insecticide Treatments

One test group received no insecticide. One group received boluses at the recommended dose of 100 to 110 milligrams per kilogram of animal weight on December 10, 1958. These calves were weighed to an accuracy of ± 2.5 pounds and dosages were adjusted to the closest quarter bolus.

Six groups received an unpelleted, molassized supplement feed into which the insecticide formulation was carefully blended by the feed formulators at the desired level of insecticide intake based on the weight of the group of calves at an allowance of 2 pounds of supplement feed per calf per day. The insecticides were fed at two total doses of 105 and 180 milligrams per kilogram and three daily doses of 5, 10 and 15 milligrams per kilogram. Thus two groups received 5 milligrams per kilogram daily for 21 and 36 days respectively; two groups received 10 milligrams per kilogram daily for 11 and 18 days respectively; and two groups received 15 milligrams per kilogram daily for 7 and 12 days respectively.

The groups to be fed Trolene were isolated in separate feed-lots on December 10, 1958, and conditioned to acceptance of untreated supplement feed for 6 days. Offering of the treated supplement feed to each of the six groups commenced on December 17, 1958. The supplement was offered to the calves in troughs each morning and no other food was given until the supplement had been eaten.

TABLE 1. — SUMMARY OF DATA ON WARBLE GRUB INFESTATIONS IN CALVES TREATED WITH TROLENE AS A BOLUS OR AS A FEED ADDITIVE AT VARIOUS DOSAGES, KAMLOOPS, B.C., 1957-1958 AND 1958-1959

	Season 1957-1958		Season 1958-1959						
	0	250	0	105			180		Supple- ment
				105 Bolus	5 21 Supple- ment	10 11 Supple- ment	15 7 Supple- ment	5 36 Supple- ment	
Total dose, mg./kg.									
Daily dose, mg./kg.	0	10	0						
Duration of treatment, days	0	25	0						
Method of administration	0	Supple- ment	0						
Number of calves	30	29	29	30	27	26	27	28	29
Number of calves infested	29	5	27	19	24	18	20	13	7
Mean number grubs per calf	43.3	0.6	37.1	6.3	17.3	8.8	12.2	9.9	4.3
Range of grubs per calf	0-229	0-7	0-176	0-38	0-68	0-52	0-198	0-72	0-8
Mean number of grubs, transformed*	1.35	0.10	1.35	0.58	0.96	0.59	0.60	0.53	0.16
S.E. of difference from control		0.119		0.139	0.148	0.153	0.149	0.161	0.072
Mean percentage reduction, transformed		93		83	63	82	75	85	95

*By the formula $\log (x + 1)$

Collection of Warble Grubs

All detectable hypodermal stage grubs were removed from all the calves by squeezing at weekly intervals from the beginning of appearance in the hypodermal stage until the number collected from the untreated group had averaged less than 0.5 grubs per animal on each of 2 successive weeks. The collection period consisted of nine collections made weekly from January 19, 1959, to March 16, 1959, inclusive.

Analysis of Data

The data were transformed logarithmically before statistical analysis because of the skewness of the distribution of the grub counts.

RESULTS AND DISCUSSION

No boluses were regurgitated after administration. All the feed-test calves appeared to eagerly accept the treated supplement feed. Minor transitory toxicity-symptoms were observed in approximately 20 per cent of the bolus-treated calves. This may appear to indicate that low-level daily dosage of Trolene is less toxic than a single high dosage but more data are required before such a conclusion can be made.

Summarized data for each of the test groups appear in Table 1. The data for the 1957-1958 feed test conducted in the same ranch herd (7) are also presented in Table 1 to extend the comparison of dosage levels.

Table 1 shows that within the range of 105 and 250 milligrams per kilogram increases in total dose of insecticide administered in supplement feed resulted in greater reductions in numbers of grubs. Rich and Ireland (7) and others have shown that within the range of 75 to 110 milligrams per kilogram increases in bolus dose did not give greater reductions. Table 1 also shows that at daily dosages of 10 and 15 milligrams per kilogram and total dosage of 180 and 250 milligrams per kilogram, feed treatments reduced the numbers of grubs significantly more than bolus treatment. Thus it would appear that feed treatment at these higher dosages is a more efficient control for warbles than bolus treatment. Furthermore, feed treatment eliminates the special equipment and special handling of animals that are required for bolus treatment.

However, Table 1 also shows that survival of grubs varied greatly within the test groups. Calves with very high infestations occurred in all groups except the 1957-1958 group and the 1958-1959 group treated at 15 milligrams per kilogram daily for 12 days. Of 330 grubs collected from 27 calves offered feed treatment at 15 milligrams per kilogram daily for 7 days, 198 or 60 per cent were collected from one calf. Similarly, of 124 grubs collected from 29 calves offered feed treatment at 10 milligrams per kilogram daily for 18 days, 88 or 70 per cent were collected from one calf. The highest individual infestation in 29 untreated calves was 176 or 16 per cent of the 1,077 grubs collected from the group. From these data it would appear that the intake of insecticide was not uniform within the groups of calves. Since the insecticide was uniformly blended into the supplement feed, it appears logical to assume that some calves, while appearing to feed freely, were consistently slow feeders and in consequence received very low dosages of insecticide.

The problem of variation in cattle feeding behaviour is not new to animal science. In a study of 10 pairs of monozygotic cattle twins, Hancock (3) found high variations in food intake per unit of feeding time that were apparently inherited characteristics. In a review of many published reports, Tribe (8) found high variations in selection of food components by individual animals when free choice of a variety of feeds was permitted. After a 3-year study of calf-feeding behaviour, Mayhew (5) concluded that much of the inconsistency occurring in published reports of tests of free-choice feeding of phenothiazine-salt mixture for warble grub and intestinal parasite control was due to inconsistency in animal feeding behaviour.

The factor is an important one. Table 1, supported by data from other workers, shows that the feed treatment method is a useful one. However, only the 1957-1958 test reduced the infestation below an average of 1 grub per calf and such a level has been observed by the writer to occur annually in apparently self-perpetuating infestations in two semi-isolated herds where grub treatment has never been applied. Therefore, the level of grub survival in the treated groups is disquieting and studies of its causes, and its significance in perpetuation of infestations, should be considered. In the meantime insecticide treatment should be so planned as to produce the highest possible reductions in the numbers of grubs. The higher insecticide requirements of the more effective feed treatments offset the economies in labour and equipment that the method shows over bolus treatment; nevertheless until additional information is available, the higher grub reductions produced would appear to justify the increased expenditure.

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NOTE ON A RAPID TEST FOR CHROMIC OXIDE IN FECES

It is often desirable in studies on digestibility and rate of passage to know at the time of collecting the feces whether or not chromic oxide is present. Most quantitative methods for determining chromic oxide in feces require considerable time, especially when wet material is involved (1, 2). A rapid qualitative test is described below for detecting trace amounts of chromic oxide in either wet or dry feces.

If using wet feces, approximately 1 gram is placed in the capsule of a Parr oxygen bomb with 1 millilitre of ethyl alcohol. Upon ignition, part of the ash is found in the form of small translucent beads approximately $\frac{1}{2}$ to 1 millimetre in diameter. The beads are tinted green if traces of chromic oxide are present, the intensity of the green colour depending upon the chromic oxide content. Dry feces are pelleted and ignited in the usual manner without alcohol.

The sensitivity of the method was checked using wet and dry cow feces to which known amounts of chromic oxide had been added. It was also checked using feces from cows fed chromic oxide in which the concentration of the indicator had been estimated by a conventional quantitative technique (1). The method required only 5 minutes and could detect as little as 0.02 milligrams of chromic oxide per gram of wet or dry feces. This was found to be below the limit of positive identification by the quantitative technique.

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Erratum:

In the June, 1959, issue of the "*Canadian Journal of Animal Science*", in the paper by Weintraub, Rich and Thompson, entitled "Timing the treatment of cattle with Trolene for systemic control of the cattle grubs, etc.", paragraph 3 on page 55 should have read:

This was done by calculating a range of mortalities (Table 1) for each treatment, which gave a more realistic measure of cattle grub control by Trolene. The following calculations* were used:

$$S_p = \frac{1}{n} \sqrt{S_m^2 + p^2 S_n^2}$$

where m and n are means of the treated and untreated grubs, respectively; $p = m/n$ and S_p , S_m and S_n are the respective standard errors of the ratio and of the corresponding means.